

iGEM2016 – Microbiology – BMB – SDU

Project type: Plastic Project title: characterizing and optimizing PHB production Sub project: 1. Extraction of PHB with different methods	Creation date: 2016.10.16 Written by: Jakob Rønning Performed by: Joel Mario Vej-Nielsen & Jakob Rønning
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1. SOPs in use.

SOP0031_v1 – TB media

SOP0001_v1 - ON culture of *E. coli*

SOP0032_v1 – Ethyl acetate PHB recovery

SOP0033_v1 – Hypochlorite and triton x-100 PHB recovery

SOP0034_v1 – Chloroform PHB recovery

SOP0044_v1 – Hypochlorite PHB recovery

2. Purpose.

Comparison of different extraction methods of biobricks.

3. Overview.

Day	SOPs	Experiments
1	SOP0031	TB media
2	SOP0001	ON culture
3	SOP0032 SOP0034	Ethyl acetate PHB recovery Chloroform PHB recovery
4	SOP0033 SOP0044	Hypochlorite and triton x-100 PHB recovery Hypochlorite PHB recovery

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Ethyl acetate		AppliChem	Anne Mette, RT	Handle in fume closet
Destilled water			Micro storage	
50 mL falcon tubes		Contact Lab-manager	Micro storage	
Acetone				Handle in fume closet
100 – 200 mL flask			Micro storage	
250 or 500 mL centrifuge tubes		Incubation room (class 1) – V18-404b-0		
Triton X-100	1% v/v in PBS		Micro storage	
PBS				
Sodium hypochlorit	13% v/v with pH of 12,3		Micro storage	
Destilled H ₂ O				
Ethanol	70 %			
Acetone		AppliChem	Anne Mette, RT	Handle in fume closet
chloroform		Contact Lab-manager	Micro storage	Handle in fume closet
Freezing tubes		Contact Lab-manager	Micro storage	
Distilled water				
Tryptone			Chemical room	
Yeast extract			Chemical room	
Glycerol			Chemical room	
1L media bottle			Micro storage	
10 x TB phosphate	2.31 g KH ₂ PO ₄ and 12.54 g K ₂ HPO ₄ in 90 ml H ₂ O, adjust to 100 ml with H ₂ O.		Chemical room	

5. Other

Primer to PCR dilution:

6. Experiment history.

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
16.09.22	SOP00031_v1 TB media	3 L of TB was produced
16.09.23	SOP0001 ON culture	Six times 200 mL TB was inoculated with K2018000
16.09.30	SOP0034 Chloroform PHB recovery	Triplets was made
16.09.30	SOP0032 Ethyl acetate PHB recovery	Triplets was made
16.10.03	ON culture	Six 200 mL TB was inoculated with K2018000
16.10.05	SOP0033 Triton x- 100 and hypochlorite PHB recovery	Triplets was made, each gram was purified with 40 ml 7 % hypochlorite, to make sure that the extraction worked out fine.
16.10.05	SOP0044 Hypochlorite PHB extraction	Triplets was made, each gram was purified with 40 ml 7 % hypochlorite, to make sure that the extraction worked out fine.

7. Sample specification.

Sample name	Sample content	From	Used for / Saved where

8. Remarks on setup

9. Results and conclusions.

All extraction methods provide PHB.

10. Appendixes